Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Initially, applicants would like to note that claims 1, 6, 11, 54, 66, and 67 have been amended; claims 2-5, 12-15, and 64-65 have been canceled without prejudice; and new claims 68-82 have been introduced. Claims 1, 6, 11, 16, 48, 49, 51-54, 66-82 are pending, and of these claims 48, 49, 52, and 54 have been allowed and claim 16 has been objected to but otherwise indicated to contain allowable subject matter.

With respect to the claims amendments, applicants submit that descriptive support is provided in the specification for the amendments and, therefore, no new matter has been added. Claim 1, in particular, has been amended to now recite a complex that comprises "heme binding protein complexed with a porphyrin." Descriptive support for the complex as presently claimed appears at page 18, line 24 to page 19, line 3. Further support in the specification has been identified in the accompanying Declaration of Maqsudul Alam, Ph.D. Under 37 C.F.R. § 1.132 ("Alam Declaration") at ¶¶ 5-6. When the HemAT-*Hs* and HemAT-*Bs* proteins taught in the present application were expressed in *E. coli* and purified, the media in which the host cells were grown contained a heme complex precursor, hemin, which allowed the host cells to produce the HemAT-*Hs* and HemAT-*Bs* proteins complexed with a heme molecule. Alam Declaration ¶ 5. The heme precursor increases the production of protoporphyrin IX, thus drastically enhancing the proper folding of the heme protein in host *E. coli*. *Id.* The use of a heme synthesis precursor as a culture media additive for the production of a functional heme complexed protein was well known at the time the present invention was made, as shown, for example, by the addition of hemin for the production of an active human hemoglobin tetramer. *Id.* The use of such media for culturing heme complexed proteins was so routine that a skilled scientist, having read the present patent application, would have understood from the teaching of the application that the recombinantly expressed proteins HemAT-*Hs* and HemAT-*Bs* had been cultured with heme precursors to form a protein-heme complex. Alam Declaration ¶ 6. This is evidenced by four characteristics of the complexes that are disclosed in the present application. *Id.* First of all, a visible consequence of forming the protein-heme complex is that a solution containing the complex will be reddish brown in color. *Id.* This is due to the iron molecule that lies at the core of the heme molecule. *Id.* This feature is noted at page 22, lines 32-33, of the present application, which recites that the partially purified HemAT-*Hs* and HemAT-*Bs* proteins are

described as a having a "brown red supernatant," indicating the presence of heme complexed with the protein. *Id.* Secondly, the spectral properties of proteins containing a heme molecule were exhibited by the isolated complex of the present patent application. *Id.* It is well known that when a protein contains a heme molecule, that protein in its oxygenated form will typically display absorption band maxima that are similar to those of other heme binding proteins (i.e., those lacking a signal transducer domain) (*see* Example 4 at pages 24-25). *Id.* The purified HemAT-*Hs* complexed with heme exhibited the following absorption band maxima: Soret band at 406 nm, α-band at 578 nm, and β-band at 538 nm (*see* Example 4 at page 24, lines 20-22, and Figure 4A). *Id.* Thirdly, a pyridine hemochrome assay performed on the complexes confirmed that the "heme group of both HemAT-*Hs* and HemAT-*Bs* ...[is the] ... b-type (*see* Example 4 at page 24, line 32 to page 25, line 1). *Id.* The fourth piece of evidence is the spectral characteristics of the complexes in response to sodium dithionite, which deoxygenates an oxygen-bound heme molecule, and subsequent exposure of the deoxygenated complex to atmospheric oxygen, which causes reversion to the previously noted absorption pattern (*see* Example 4 at pg. 24, lines 24-29, and Figures 4B, 4D). *Id.* From the foregoing, it is clear from the data provided in the present patent application that the isolation of HemAT-*Hs* and HemAT-*Bs* proteins as taught in the patent application produced in each instance an isolated heme-binding protein complexed to a heme molecule.

From the foregoing, the present application provides descriptive support for the presently claimed subject matter.

The rejection of claim 64 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is rendered moot by the cancellation of this claim without prejudice.

The rejection of claims 1-5, 11-15, 53, and 64-67 under 35 U.S.C. § 112 (1st para.) for failure to meet the written description requirement is respectfully traversed in view of the above amendments and the following remarks.

Claim 1 has been amended to recite "[a]n isolated complex comprising a heme binding protein complexed with a porphyrin, wherein said complex reversibly binds oxygen with a low affinity and wherein said protein comprises a heme binding domain that associates with the porphyrin and an aerotaxis signaling domain."

As noted above, the present application identifies two species of heme binding proteins that have a heme binding domain and an aerotaxis signaling domain, HemAT-*Hs* and HemAT-*Bs*, with the proteins being complexed to a porphyrin such as heme. The

specification further identifies regions within the heme binding domain that are conserved among globin-type proteins.

For these reasons, and the reasons noted above, applicants submit that the two species disclosed in the specification provide adequate written descriptive support for the presently claimed complex. Therefore, the rejection is improper and should be withdrawn.

The rejection of claims 1-6 under 35 U.S.C. § 102(b) as anticipated by Zhang is respectfully traversed in view of the above amendments and the following remarks.

It is the position of the U.S. Patent and Trademark Office ("PTO") that Zhang anticipates the present invention by disclosing a protein ("HtB") from *H. salinarium* having all the properties of the protein of claim 1-6 of the present invention (page 7 of office action). Applicants submit, as demonstrated below, based on the accompanying Alam Declaration that the presently claimed complex is distinct of the HtB protein described in Zhang.

Zhang relates to the identification of a putative signal transduction gene family from *H. salinarium*, and discloses the partial purification of several methyl-accepting taxis halobacterial proteins of that family. Alam Declaration ¶ 7. Although some physical properties of the HtB protein were disclosed in Zhang, there is no disclosure in Zhang that HtB was a heme-binding protein and, therefore, could form a complex with heme. *See id.* In fact, at the time of publishing Zhang, the function of HtB was not known. *Id.* Given that it was unknown that HtB was a heme-binding protein, heme complex precursors were never added to the culture media when growing the bacterium used for the *H. salinarium* signal transducer protein localization studies as reported Zhang. *Id.* As a result, none of the partially purified *H. salinarium* signal transducer proteins disclosed in Zhang were complexed with a heme molecule. *Id.* From all of the above, it is clear that the HtB *H. salinarium* signal transducer protein disclosed in Zhang is not in the form of a complex as presently claimed in the above-identified patent application.

Because Zhang fails to teach or suggest the presently claimed invention, the rejection of claims 1-6 as anticipated by Zhang is improper and should be withdrawn.

The rejection of claims 1, 3-5, 11, 13-15, and 64 under 35 U.S.C. § 102(b) as anticipated by U.S. Patent No. 5,635,207 to Grinstaff et al. ("Grinstaff") is respectfully traversed. It is the position of the PTO that Grinstaff anticipates the claimed invention because Grinstaff teaches a method of preparing a blood substitute comprising myoglobin. However, Grinstaff does not teach or suggest the formation of a complex as presently

claimed. In particular, myoglobin, unlike the heme binding protein included in the presently claimed complex, fails to contain an aerotaxis signaling domain. Thus, Grinstaff cannot anticipate the claimed invention. For this reason, the rejection of claims 1, 3-5, 11, 13-15, and 64 as anticipated by Grinstaff should be withdrawn.

The rejection of claims 1, 3-5, 11, 13-15, and 64 under 35 U.S.C. § 102(b) as anticipated by Sugimoto et al., "Myoglobin Mutants Giving the Largest Geminate Yield in CO Rebinding in the Nanosecond Time Domain," *Biophysical Journal* 75:2188-2194 (1998) ("Sugimoto") is respectfully traversed. Sugimoto relates to recombinant mutant sperm whale myoglobin proteins isolated and purified using an *E. coli* expression system. It is the position of the PTO that the myoglobin disclosed by Sugimoto is identical to the protein of the claimed invention. However, Sugimoto does not teach or suggest the formation of a complex as presently claimed. In particular, sperm whale myoglobin, unlike the heme binding protein included in the presently claimed complex, fails to contain an aerotaxis signaling domain. Thus, Sugimoto cannot anticipate the claimed invention. For this reason, the rejection of claims 1, 3-5, 11, 13-15, and 64 as anticipated by Sugimoto is improper and should be withdrawn.

The rejection of claims 1, 3-5, 11, 13-15, and 64 under 35 U.S.C. § 102(b) as anticipated by Zhao et al., "A Double Mutant of Sperm Whale Myoglobin Mimic the Structure and Function of Elephant Myoglobin," *J. Biol. Chem.* 270(35):20763-20774 (1995) ("Zhao") is respectfully traversed. Zhao relates to a L29F/H64Q double mutant of sperm whale myoglobin having a low O_2 binding affinity, where the mutant proteins are recombinant sperm whale proteins obtained by using a bacterial expression system. Zhao does not teach or suggest the formation of a complex as presently claimed. In particular, the mutant sperm whale myoglobin of Zhang, unlike the heme binding protein included in the presently claimed complex, fails to contain an aerotaxis signaling domain. Thus, Zhao cannot anticipate the claimed invention. For this reason, the rejection of claims 1, 3-5, 11, 13-15, and 64 as anticipated by Zhao is improper and should be withdrawn.

The rejection of claims 1-6 and 66-67 under 35 U.S.C. § 103(a) for obviousness over Zhang in view of Yao et al., "Primary Structure of an Archaebacterial Transducer, a Methyl-Accepting Protein Associated with Sensory Rhodopsin I," *Proc. Natl. Acad. Sci. U.S.A.* 89:11915-11919 (1992) ("Yao I") and Yao et al., "Identification of Distinct

Domains for Signaling and Receptor Interaction of the Sensory Rhodopsin I Transducer, HtrI," *J. Bacteriology* 176(22):6931-6935 (1994) ("Yao II") is respectfully traversed in view of the amendments above and the remarks below.

The teaching and the deficiencies of Zhang, as it relates to the presently claimed complex of claim 1, are disclosed above. Yao I relates to the cloning and sequencing of a 97-kDa methyl-accepting protein that acts as a signal-transducer for a phototaxis receptor ("SR-I) in the archeabacterium *H. salinarium*. Yao II discloses that the putative transducer protein, HtrI, of *H. salinarium* contains at least two domains that are involved in the phototaxis response of the archeabacteria. Yao I and Yao II do not overcome the above-noted deficiencies of Zhang, either individually or in combination.

For this reason, the rejection of claim 1-6 and 66-67 for obviousness over Zhang in view of Yao I and Yao II is improper and should be withdrawn.

The objection to claim 16 is overcome by the above amendments to claims 1 and 11, and should therefore be withdrawn.

In view of all of the foregoing, applicant submits that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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